JOURNAL OF VIROLOGY, July 1995, p. 4582–4586 Vol. 69, No. 7
0022-538X/95/$04.00+0

Aberrant Gag Protein Composition of a Human Immunodeficiency Virus Type 1 *vif* Mutant Produced in Primary Lymphocytes

MALGORZATA SIMM,[1] MUHAMMAD SHAHABUDDIN,[1] WEI CHAO,[1]
JONATHAN S. ALLAN,[2] AND DAVID J. VOLSKY[1]*

Molecular Virology Laboratory, St. Luke's-Roosevelt Hospital Center and College of Physicians & Surgeons, Columbia University, New York, New York 10019,[1] and Department of Virology & Immunology, Southwestern Foundation for Biomedical Research, San Antonio, Texas[2]

Received 19 September 1994/Accepted 23 March 1995

Productive, spreading infection of peripheral blood lymphocytes (PBL) with human immunodeficiency virus type 1 (HIV-1) requires the viral protein Vif. To study the requirement for *vif* in this system, we infected PBL with a phenotypically complemented HIV-1 clone mutated in *vif*. Progeny virus was produced which was noninfectious in PBL but replicated in SupT1 cells. Analysis of metabolically labeled proteins of sedimentable extracellular particles made in PBL by radioimmunoprecipitation with either serum from a patient with AIDS or a monoclonal antibody reactive with HIV-1 Gag proteins revealed that *vif*-negative but not wild-type particles carry higher levels of p55, p41, and p38 Gag-specific proteins compared with those of p24. Similar results were obtained with sucrose-purified virions. Our data indicate that *vif* plays a role in Gag protein processing or in incorporation of processed Gag products into mature virions. The presence of unprocessed precursor Gag polyprotein (Pr55^{gag}) and other Gag processing intermediates in PBL-derived *vif*-negative extracellular particles may contribute to the reduced infectivity of this virus.

The Vif protein of human immunodeficiency virus type 1 (HIV-1) determines HIV-1 infectivity as a function of the cell type producing the virus (7, 8, 24, 29, 33). Many cultured T-cell lines permit infection by *vif*-negative HIV-1 (7, 8, 15, 24, 28, 29, 33), but peripheral blood lymphocytes (PBL) are considered nonpermissive to this virus (3, 8, 19, 33). Research indicates that Vif acts late in the virus life cycle (3, 4, 23, 33) and that in nonpermissive cells, Vif may modify progeny virions to increase their infectivity (7, 8, 28, 29, 33). The nature of these modifications is unknown. Sakai et al. reported that HIV-1 particles produced in the absence of *vif* have reduced levels of gp120, suggesting that *vif* may affect the insertion of viral glycoproteins into viral envelopes (24). Others detected no differences between HIV-1 proteins produced during infection of various cell lines in the presence or absence of functional *vif* (2, 7, 8, 33). A recent study by electron microscopy revealed that cores of mature *vif*-mutant virions made in CEM and Jurkat cell lines, which are semipermissive to replication of *vif*-negative virus (8), have a nonhomogeneous morphology compared with wild-type virus, and the authors suggested that Vif has a role during packing of the viral nucleoprotein core (10). The presence of a *vif*-related nucleocapsid defect can also be inferred from studies which showed that in target cells that require *vif* for virus replication, *vif*-negative HIV-1 can fuse with and enter cells (16, 33) but fails to synthesize viral DNA efficiently (27, 33).

The aim of the present work was to explore the possibility that viral particles made in nonpermissive cells in the absence of *vif* have aberrant *gag* gene products, in correlation with other structural (10) and functional (28, 33) defects. We have chosen for these studies virions produced in PBL because, in contrast to many transformed cell lines, primary lymphocytes seem to have minimal endogenous *vif*-complementing activity (3, 8, 19, 33). To produce *vif*-negative progeny HIV-1 in PBL, we took advantage of the finding that *vif* mutants passaged in certain transformed cells are phenotypically complemented; that is, they replicate like wild-type viruses during the first cycle of infection in nonpermissive cells (8, 28). Infected cells were metabolically labeled with a mixture of [^{35}S]methionine and [^{35}S]cysteine, and the newly synthesized proteins present in sedimentable extracellular particles collected from culture supernatants were analyzed by immunoprecipitation and sodium dodecyl sulfate (SDS)-gel electrophoresis as previously described (25). The *vif*-negative mutant used in the present studies was constructed on the background of the infectious molecular clone NL4-3, which carries a full complement of functional viral genes (1). Briefly, the 621-bp *Nde*I-*Eco*RI fragment of NL4-3 *vif* was replaced with the corresponding 586-bp fragment of N1T-E *vif*, yielding the NL4-3ΔVif clone, which contains a 35-bp deletion in *vif* that is responsible for the *vif*-negative phenotype of N1T-E (23). This clone is called ΔVif in the text. The wild-type (WT) control clone was constructed from NL4-3 similarly but with the corresponding 621-bp *Nde*I-*Eco*RI *vif* fragment from N1T-A, a coisolate of N1T-E which has an intact *vif* gene (23). The ΔVif and WT clones are isogenic except for their respective *Nde*I-*Eco*RI fragments in *vif*. The functionality of the new constructs was tested, as previously described (17), in transient-expression assays following transfection of viral DNA into rhabdomyosarcoma (RD) cells. Both WT and ΔVif produced 200 to 300 ng of viral p24 antigen per ml 3 days after transfection (data not shown). The RD cell-derived viruses were standardized by their p24 content and tested for infectivity in PBL and SupT1 cells (27), the latter being known to partially complement the *vif* defect (33) (Fig. 1). Cells were infected with virus at 1 pg of p24 per cell, which in our hands is equivalent to a multiplicity of infection of 1 for WT viruses (17). For this and subsequent experiments, PBL

* Corresponding author. Mailing address: Molecular Virology Laboratory, St. Luke's-Roosevelt Hospital Center, 432 W. 58th St., New York, NY 10019. Phone: (212) 582-4451. Fax: (212) 582-5027. Electronic mail address: djv4@Columbia.edu.

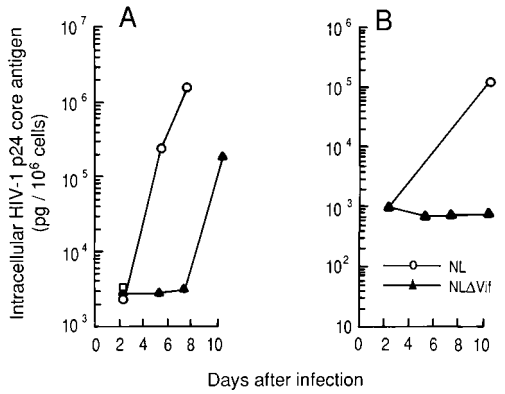

FIG. 1. Infection of SupT1 cells (A) and PBL (B) by ΔVif HIV-1. ΔVif and WT viruses were produced by DNA transfection into RD cells; supernatants were standardized by their p24 content and used to infect PBL and SupT1 cells at 1 pg of p24 per cell as described in the text.

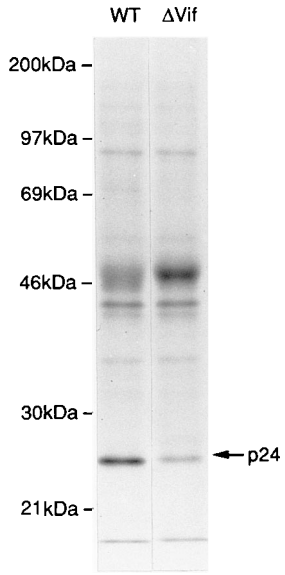

FIG. 2. Viral proteins in sedimentable viral particles collected from PBL infected with ΔVif or WT as determined by radioimmunoprecipitation with a serum sample from a patient with AIDS. See the text for details.

were isolated from heparinized venous blood of HIV-1-negative volunteers by density gradient centrifugation with Ficoll-Hypaque and were stimulated and cultured in RPMI 1640 medium supplemented with fetal bovine serum, antibiotics, and interleukin-2. SupT1 cells were cultured as above without interleukin-2.

As shown in Fig. 1, the WT and ΔVif viruses made in RD cells were highly productive in SupT1 cells, although the peak of production of the *vif* mutant was shifted by 1 week, reflecting the time needed to generate phenotypically complemented virions from the original inoculum (7, 16, 31). In contrast, and consistent with other reports (3, 8, 19, 33), no significant p24 levels were found in culture supernatants of PBL infected with ΔVif. These results show that our WT and ΔVif mutant viral clones display infection phenotypes typical for these viruses (2, 8, 33).

In two subsequent infections of PBL, we used ΔVif or WT viral stocks prepared and subjected to titer determination in SupT1 cells. To maximize ΔVif virus protein production during single-cycle infection in PBL, the virus was used at a multiplicity of infection of 2, or 2 pg of p24 per cell. No significant cytolysis was observed. WT virus was used at a multiplicity of infection of 1. Infected cells were metabolically labeled with [^{35}S]methionine and [^{35}S]cysteine for 30 h (42 to 72 h after infection), and culture supernatants were collected on day 3 and subjected to centrifugation at 200,000 × *g* for 4 h to pellet supernatant virus particles. The pellets obtained from 3.5 × 10^6 cells in each system were lysed and subjected to immunoprecipitation with a serum sample from a patient with AIDS followed by electrophoresis through an SDS–10% polyacrylamide gel and autoradiography as previously described (25). The analysis of labeled HIV-1 proteins present in pelletable particles released by PBL in a representative experiment is shown in Fig. 2. As the autoradiogram shows, similar major proteins were precipitated from ΔVif and WT virion lysates with the serum sample; they include the 17-, 24-, and 55-kDa bands, which probably represent viral Gag p17, p24, and Gag precursor p55 polyproteins, respectively (9, 18, 35). A protein migrating with an apparent molecular mass of 120 kDa (probably gp120) was seen upon long exposure of the autoradiogram (data not shown). This result is consistent with previous analyses of *vif* mutant and WT viral proteins produced in transformed cells (2, 8, 33). However, the respective ratios of 55- to 24-kDa proteins in WT and ΔVif particles produced in PBL were reversed; the 55-kDa band significantly predominated over the 24-kDa band in ΔVif particles but was only a minor component in WT virions (Fig. 2). This finding was reproduced in three experiments with PBL from different donors and different stocks of ΔVif and WT viruses, and it indicated that ΔVif virus made in PBL had a different protein composition from WT virions produced under the same conditions.

We next sought to confirm that the predominant 55-kDa band seen in the ΔVif lane in Fig. 2 is a Gag-related protein, probably the 55-kDa Gag precursor polyprotein. In the normal process of HIV-1 virion production and maturation, the core (Gag) structural proteins are initially synthesized as two polyprotein precursors, Pr55^{gag}, encoded by the *gag* gene, and Pr160$^{gag\text{-}pol}$, encoded by a combined *gag-pol* reading frame created by a ribosomal frameshift (11, 18, 31). Near the time of virion budding, Pr55^{gag} is cleaved by the viral protease into p17 (matrix), p24 (core), p9 (nucleocapsid), and p6; the cleavage of Pr160$^{gag\text{-}pol}$ yields p24, p17, and truncated p9 (9, 11, 20, 31, 34), in addition to *pol* gene products. Thus, mature virions are not likely to contain significant amounts of precursor Gag proteins. We infected PBL with ΔVif and WT viruses essentially as described above and analyzed viral proteins in extracellular pelleted particles by immunoprecipitation with monoclonal SIV-AG3.0 antibody to SIV_{agm} Gag proteins. This antibody cross-reacts with HIV-1 p24, precursor proteins Pr55^{gag} and Pr160$^{gag\text{-}pol}$, and the intermediate Gag precursor-processing products, such as p38 (3a). In this experiment, we compared Gag virion proteins detected with the AG3.0 antibody in pelleted particles after infected cells had been labeled for either 30 h (42 to 72 h postinfection) or 8 h (64 to 72 h postinfection); supernatants from 3.5 × 10^6 cells were collected for analysis 3 days after infection in each case. The results are shown in Fig. 3. The pattern of Gag-specific proteins produced under the 30-h labeling conditions confirmed the result obtained with serum from a patient with AIDS (Fig. 2), namely, that pelleted ΔVif particles contained a large excess of a 55-kDa protein over p24 while WT particles produced under the same conditions carried predominantly p24 and almost no p55 (30-h panel in Fig. 3). Since the SIV-AG3.0 antibody recognizes specifically viral Gag proteins, the 55-kDa component is most prob-

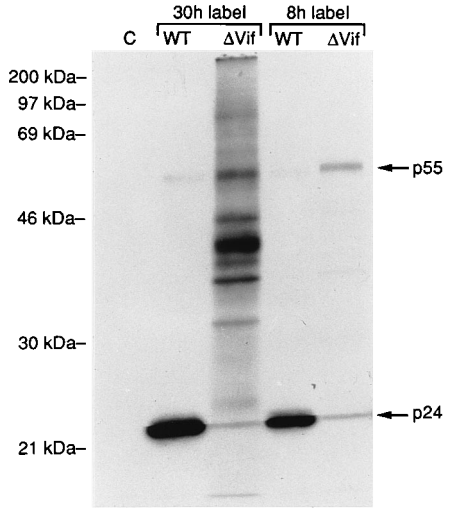

FIG. 3. Viral Gag-related proteins in pelleted viral particles collected from PBL infected with ΔVif or WT viruses as determined by radioimmunoprecipitation with a monoclonal anti-Gag antibody.

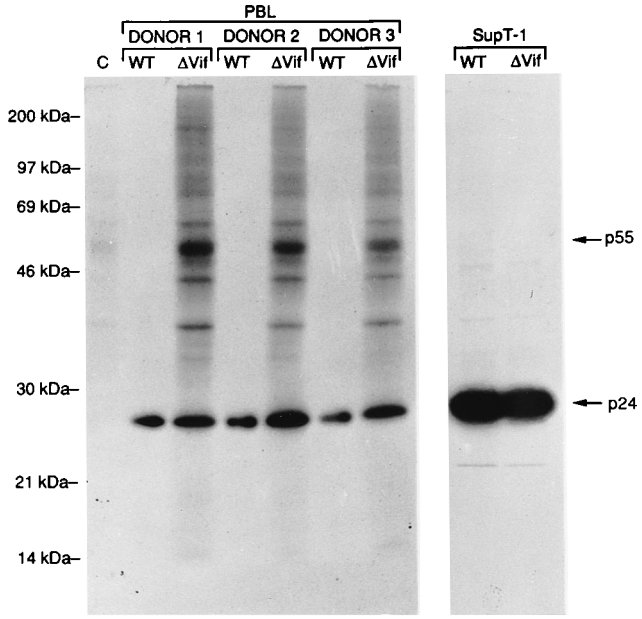

FIG. 4. Viral proteins in pelleted viral particles obtained from PBL or SupT1 cells infected with ΔVif or WT as determined by radioimmunoprecipitation with a monoclonal anti-Gag antibody.

ably an unprocessed 55-kDa Gag precursor polyprotein ($Pr55^{gag}$) of HIV-1 present in pelleted ΔVif particles. The low levels of the 55-kDa protein in WT virions are consistent with the notion that under normal conditions much of the proteolytic processing of Gag precursor proteins occurs prior to or during budding (9, 11, 20, 21, 31, 34); as a consequence, secreted virions carry predominantly processed Gag products. The 30-h labeling conditions also revealed several major Gag-related proteins in ΔVif particles which were of intermediate molecular sizes between p55 and p24, notably, p41 and p38. They probably represent the Gag polyprotein-processing intermediates often seen in virus-like particles generated by *gag* or *gag-pol* expression vectors (6, 14, 15, 25, 30, 34) or in pelletable virions produced in the presence of an HIV-1 protease inhibitor (13). The protein with an approximate molecular mass of 41 kDa was most prominent in the gel shown in Fig. 3, but the ratio among the different Gag intermediates precipitated by the AG3.0 antibody from ΔVif particles differed from experiment to experiment (cf Fig. 4 [below]). The extracellular ΔVif particles labeled for the last 8 h of incubation contained Gag-specific $Pr55^{gag}$ and p24 proteins but no detectable processing intermediates (8-h label in Fig. 3). As in the 30-h labeling, p55 predominated over p24 in ΔVif particles while WT virions tested in parallel contained p24 only. This result indicates that $Pr55^{gag}$ is a major Gag component of extracellular pelletable ΔVif particles toward the end of the predicted single-cycle infection. The reasons for the absence of other atypical Gag-related products during this period of infection are unclear. No proteins were detected in immunoprecipitates of ΔVif virions prepared in parallel with mouse immunoglobulin G as a control (lane C in Fig. 3). These results show that sedimentable ΔVif but not WT particles produced in PBL contain high levels of unprocessed $Pr55^{gag}$, as well as several smaller Gag-specific proteins not normally found in extracellular WT HIV-1.

To test the reproducibility of the *vif*-specific defect of Gag-related proteins in ΔVif made in PBL, we infected cells from three different donors with ΔVif and WT viruses, metabolically labeled cells for 30 h, and analyzed newly synthesized proteins in pelleted extracellular particles by using the SIV-AG3.0 antibody 3 days after infection as described above (Fig. 4). In this analysis PBL-derived samples were standardized by their content of radioactive p24 on a gel, resulting in high backgrounds in ΔVif lanes. We also compared the Gag-related proteins in ΔVif and WT made in semipermissive SupT1 cells (33), which were labeled and analyzed similarly to PBL-made viruses (SupT1 panel in Fig. 4). As shown in Fig. 4, all three PBL infections yielded sedimentable ΔVif particles which contained high levels of $Pr55^{gag}$ and variable amounts of atypical Gag-related proteins. In this experiment, the p55 protein was a predominant atypical Gag-specific component in ΔVif particles in all three cultures. *vif*-negative particles obtained from donor 1 cells also contained a clearly detectable protein of approximately 160 kDa, which may represent $Pr160^{gag\text{-}pol}$ (Fig. 4). Only p24 was seen in WT virions produced and labeled under the same conditions. In contrast to PBL-derived ΔVif, mutant virus produced by SupT1 cells infected with the same viral stock and analyzed in parallel contained little or no unprocessed $Pr55^{gag}$, resembling in this respect WT virions produced in PBL and SupT1 (Fig. 4). We conclude that a defect resulting in the presence of large amounts of unprocessed $Pr55^{gag}$ in extracellular virions is specific for PBL infected with ΔVif HIV-1. The absence of $Pr55^{gag}$ in extracellular ΔVif particles released by SupT1 cells (Fig. 4) may be related to *vif* complementing activity in these cells (8, 33).

To determine whether the precursor Gag polyproteins and atypical Gag-processing intermediates produced by ΔVif HIV-1-infected PBL are present in virus-like particles rather than in sedimentable particulate matter, PBL were infected and metabolically labeled as described above and virions in cell-free culture supernatants were pelleted through a 15% sucrose cushion at 200,000 × *g* for 16 h. For this experiment, ΔVif and WT viruses were produced in poorly permissive MT-2 cells (28); both viruses were used at 1 pg of p24 per cell. HIV-1 virions produced in chronically infected CR10/N1T cells (5) incubated with 1 μM HIV-1 protease inhibitor Ro 31-8959 (22) for 24 h, metabolically labeled, and pelleted through a sucrose cushion as described above served as control viral particles known to contain unprocessed $Pr55^{gag}$ (22). All three

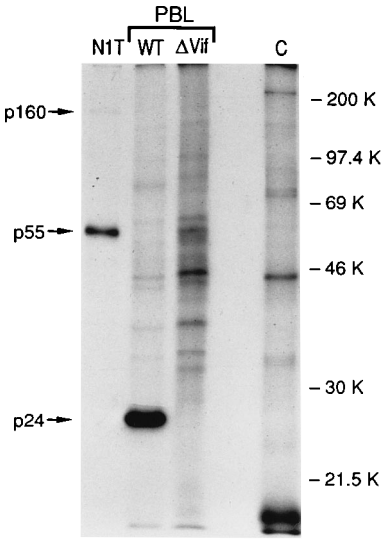

FIG. 5. Viral proteins in sucrose cushion-purified viral particles obtained from PBL infected with WT or ΔVif viruses (WT and ΔVif lanes, respectively) or from chronically infected CR10/N1T cells cultured with 1 μM Ro 31-8959 (N1T lane) as determined by radioimmunoprecipitation with a monoclonal anti-Gag antibody.

purified virion preparations were analyzed by immunoprecipitation with the AG3.0 antibody followed by SDS-polyacrylamide gel electrophoresis as described above; the results are shown in Fig. 5. As expected (13, 22), virions produced in the presence of the HIV-1 protease inhibitor contained predominantly $Pr55^{gag}$, a small amount of $Pr160^{gag\text{-}pol}$, and no processed Gag products (N1T lane in Fig. 5), while WT virions produced in PBL contained predominantly p24 and no detectable Gag precursor polyproteins (WT lane in Fig. 5). Sucrose cushion-purified ΔVif produced in PBL contained $Pr55^{gag}$ and several atypical Gag-specific proteins, notably p41 and p38, but no detectable p24 (ΔVif lane in Fig. 5). These data are consistent with the analyses of unpurified progeny HIV-1 shown in Fig. 3 and 4, and they indicate that PBL infected with ΔVif HIV-1 produced bona fide viral particles which carry unprocessed Gag proteins and Gag-processing intermediates.

To test the correlation between the observed Gag protein patterns and progeny virus infectivity, infected PBL from the experiment whose results are shown in Fig. 3 were cocultivated with uninfected PBL or SupT1 cells 3 days after infection (Table 1). PBL-derived ΔVif replicated, with a delay, in SupT1 cells but not in PBL; PBL-derived WT virus replicated in both cell types (Table 1). The results indicate that some ΔVif particles produced in PBL are infectious and can spread in SupT1 cells after cell-to-cell transmission and complementation of the *vif* defect by SupT1. SupT1 cells appear to complement the *vif* defect in ΔVif virus by allowing complete processing of $Pr55^{gag}$ prior to virus budding from cells (Fig. 4). Since no such complementation is evident in PBL and ΔVif does not spread in these cells, a causal relationship may exist between the presence of high levels of $Pr55^{gag}$ in ΔVif virus made in PBL and diminished infectivity of this mutant.

TABLE 1. Infectivity of PBL-derived ΔVif virus in PBL and SupT1 cells[a]

Time after infection (days)	Amt of HIV-1 p24 core antigen (ng/ml) in:			
	PBL/WT		PBL/ΔVif	
	PBL	SupT1	PBL	SupT1
2	125	94	3	2
4	260	707	3	0.6
8	150	470	0.3	0.3
10			0.3	0.5
15			0.1	64

[a] WT- or ΔVif-infected PBL (PBL/WT or PBL/ΔVif, respectively) from the experiment shown in Fig. 3 were cocultivated 3 days after infection with uninfected PBL or SupT1 cells at a 1:10 ratio and tested for the levels of HIV-1 p24 core antigen in culture supernatants at the indicated times.

Taken together, these results demonstrate that ΔVif HIV-1 produced in PBL contains high levels of $Pr55^{gag}$, resulting in an abnormally high $Pr55^{gag}$/p24 ratio in these particles compared with WT virions. In addition, PBL-derived ΔVif virions contain different levels of Gag-processing intermediates, notably p41 and p38, which are not normally found in WT virions. We suggest that Vif may be required in PBL for the production or assembly of progeny virions which carry only processed Gag products. It is important to note in this context that the processing of Gag and Pol precursor polyproteins in oncogenic type C retroviruses, which do not carry a *vif*-like gene, occurs only after the release of progeny virions from cells (20). HIV-1 Gag precursor polyproteins are processed prior to virion budding (12, 20). On the basis of the results presented here, this difference might be attributed to the presence of *vif*. Other investigators have suggested that ΔVif and WT virions made in poorly permissive CEM cells have similar levels of major viral structural proteins (33). However, the pelleted particles used for that analysis were standardized by their p24 antigen contents (33), which, in accordance with the data shown in Fig. 3 here, results in similar p24 protein bands on gels. The investigators did not comment on the presence or absence of unprocessed Gag products in the ΔVif virions produced in CEM cells (33). Since p24 is present in *vif*-defective particles made in peripheral blood mononuclear cells, albeit at lower levels than in WT virions (Fig. 2 and 3), our data suggest that *vif* affects the kinetics or the overall efficiency of $Pr55^{gag}$ processing by viral protease rather than acting as a protease itself. The proteolytic processing of the HIV-1 Gag-Pol precursor proteins is highly sequential and regulated (reviewed in reference 19), and, as recently shown (21), it can be influenced by other viral proteins. Moreover, even partial blocking of HIV-1 protease activity with protease inhibitors has been shown to have a significant effect on progeny virus morphology and infectivity (13). It should be noted in this context that the pattern of Gag-specific proteins found in ΔVif virions produced in PBL (Fig. 5) is reminiscent of that reported for HIV-1 particles produced under the conditions of partial inhibition of viral protease (13). Likewise, in both situations, the progeny virions are significantly less infectious than are WT viruses (13) (Table 1).

The finding that Vif may affect processing of the HIV-1 Gag precursor polyproteins was unexpected. Results of previous studies with *gag-pol* or *gag* expression vectors indicate that Gag precursor polyproteins can be processed in the absence of other viral gene products (14, 15, 26, 30, 35). However, those studies were performed with transformed cell lines which may have a *vif*-complementing activity (8, 33), and the results are not directly comparable to the data obtained here with PBL. We show here that the effect of Vif is most prominent in PBL, which are at the bottom of the "cell permissiveness" scale with respect to the susceptibility to *vif*-negative HIV-1 infection (32). The proposed effect of *vif* on $Pr55^{gag}$ processing is consistent with the recent demonstration of abnormal viral core morphology and defective viral DNA synthesis of *vif*-negative virions (10, 28, 33). The absence of functional *vif* in PBL may

also lead to atypical *pol* gene products in ΔVif particles, but this possibility was not tested in the present work. On the basis of the results shown here, we propose that the presence of unprocessed Gag polyproteins in progeny virions is responsible at least in part for the diminished infectivity of PBL-derived ΔVif virus.

We thank M. Martin for providing the NL4-3 viral clone, A. Srinivasan for providing RD cells, I. Duncan (Roche Products) for providing Ro 31-8959, G. Bentsman and M. Damsky for providing technical help, and L. Peters for typing the manuscript.

This work was supported in part by grants CA3765, HL43628, and AI35466 from the National Institutes of Health and by a grant from the Aaron Diamond Foundation, New York.

REFERENCES

1. **Adachi, A., H. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, and M. Martin.** 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. J. Virol. **59:**284–291.
2. **Adachi, A., N. Ono, H. Sakai, K. Ogawa, R. Shibata, T. Kiyomasu, H. Masuike, and S. Ueda.** 1991. Generation and characterization of the human immunodeficiency virus type 1 mutants. Arch. Virol. **117:**45–58.
3. **Akari, H., J. Sakuragi, Y,. Takebe, K. Tomonaga, M. Kawamura, M. Fukasawa, T. Miura, T. Shinjo, and M. Hayami.** 1992. Biological characterization of human immunodeficiency virus type 1 and type 2 mutants in human peripheral blood mononuclear cells. Arch. Virol. **123:**157–167.

3a. **Allan, J. S.** Unpublished results.

4. **Blanc, D., C. Patience, T. F. Schulz, R. Weiss, and B. Spire.** 1993. Transcomplementation of Vif^- HIV-1 mutants in CEM cells suggests that Vif affects late steps of the viral life cycle. Virology **193:**186–192.
5. **Casareale, D., M. Stevenson, K. Sakai, and D. J. Volsky.** 1987. A human T-cell line resistant to cytopathic effects of the human immunodeficiency virus (HIV). Virology **156:**40–49.
6. **Dru, A., M. A. Ludosky, J. Cartaund, and G. Beaud.** 1994. Cell line dependent release of HIV-like Gag particles after infection of mammalian cells with recombinant vaccinia viruses. AIDS Res. Hum. Retroviruses **10:**383–390.
7. **Fischer, A. G., B. Ensoli, L. Ivanoff, M. Chamberlain, S. Petteway, L. Ratner, R. C. Gallo, and F. Wong-Staal.** 1987. The *sor* gene of HIV-1 is required for efficient virus transmission in vitro. Science **237:**888–893.
8. **Gabuzda, D. H., K. Lawrence, E. Langhoff, E. Terwilliger, T. Dorfman, W. A. Haseltine, and J. Sodroski.** 1992. Role of *vif* in replication of human immunodeficiency virus type 1 $CD4^+$ T lymphocytes. J. Virol. **66:**6489–6495.
9. **Henderson, L. E., M. A. Bowers, R. C. Sowder II, S. A. Serabyn, D. G. Johnson, J. W. Bess, Jr., L. O. Arthur, D. K. Bryant, and C. Fenselau.** 1992. Gag proteins of the highly replicative MN strain of human immunodeficiency virus type 1: posttranslational modifications, proteolytic processings, and complete amino acid sequences. J. Virol **66:**1856–1865.
10. **Hoglund, S., A. Ohagen, K. Lawrence, and D. Gabuzda.** 1994. Role of *vif* during packing of the core of HIV-1. Virology **201:**349–355.
11. **Jacks, T., M. D. Power, F. R. Masiarz, P. A. Luciw, P. J. Barr, and H. E. Varmus.** 1988. Characterization of ribosomal frameshifting in HIV-1 *gag-pol* expression. Nature (London) **331:**280–283.
12. **Kaplan, A. H., M. Manchester, and R. Swanstrom.** 1994. The activity of the protease of human immunodeficiency virus type 1 initiates at the membrane of infected cells before the release of viral proteins and is required for release to occur with maximum efficiency. J. Virol. **68:**6782–6786.
13. **Kaplan, A. H., J. A. Zack, M. Knigge, D. A. Paul, D. J. Kempf, D. W. Norbeck, and R. Swanstrom.** 1993. Partial inhibition of human immunodeficiency virus type 1 protease results in aberrant virus assembly and the formation of noninfectious particles. J. Virol. **67:**4050–4055.
14. **Karacostas, V., K. Nagashima, M. A. Gonda, and B. Moss.** 1989. Human immunodeficiency virus-like particles produced by a vaccina virus expression vector. Proc. Natl. Acad. Sci. USA **86:**8964–8967.
15. **Krausslich, H. G., C. Ochsenbauer, A. M. Traenckner, K. Mergener, M. Facke, H. R. Gelderblom, and V. Bosch.** 1993. Analysis of protein expression and virus-like particle formation in mammalian cell lines stably expressing HIV-1 *gag* and *env* gene products with or without active HIV proteinase. Virology **192:**605–617.
16. **Ma, X.-Y., K. Sakai, F. Sinangil, E. Golub, and D. J. Volsky.** 1990. Interaction of a noncytopathic human immunodeficiency virus type 1 (HIV-1) with target cells: efficient virus entry followed by delayed expression of its RNA and protein. Virology **176:**184–194.
17. **Ma, X.-Y., P. Sova, W. Chao, and D. J. Volsky.** 1994. Cysteine residues in the Vif protein of human immunodeficiency virus type 1 are essential for viral infectivity. J. Virol. **68:**1714–1720.
18. **Mervis, R. J., N. Ahmad, E. P. Lillehoj, M. G. Raum, F. H. R. Salazar, H. W. Chan, and S. Venkatesan.** 1988. The *gag* gene products of human immunodeficiency virus type 1: alignment within the *gag* open reading frame, identification of postranslational modifications, and evidence for alternative *gag* precursors. J. Virol. **62:**3993–4002.
19. **Michaels, F. H., N. Hattori, R. C. Gallo, and G. Franchini.** 1993. The human immunodeficiency virus type 1 (HIV-1) Vif protein is located in the cytoplasm of infected cells and its effect on viral replication is equivalent in HIV-2. AIDS Res. Hum. Retroviruses **9:**1025–1030.
20. **Oroszlan, S., and R. B. Luftig.** 1990. Retroviral proteinases. Curr. Top. Microbiol. Immunol. **157:**153–185.
21. **Pettit, S. C., M. D. Moody, R. S. Wehbie, A. H. Kaplan, P. V. Nantermet, C. A. Klein, and R. Swanstrom.** 1994. The p2 domain of human immunodeficiency virus type 1 Gag regulates sequential proteolytic processing and is required to produce fully infectious virions. J. Virol. **68:**8017–8027.
22. **Roberts, N. A., J. A. Martin, D. Kinchington, A. V. Broadhurst, J. C. Craig, I. B. Duncan, S. A. Galpin, B. K. Handa, J. Kay, A. Krohn, R. W. Lambert, J. H. Merrett, J. S. Mills, K. E. B. Parkes, S. Redshaw, A. J. Ritchie, D. L. Taylor, G. J. Thomas, and P. J. Machin.** 1990. Rational design of peptide-based HIV proteinase inhibitors. Science **248:**358–361.
23. **Sakai, K., X.-Y. Ma, I. Gordienko, and D. J. Volsky.** 1991. Recombinational analysis of a natural noncytopathic human immunodeficiency virus type 1 (HIV-1) isolate: role of the *vif* gene in HIV-1 infection kinetics and cytopathicity. J. Virol. **65:**5765–5773.
24. **Sakai H., R. Shibata, J.-I. Sakuragi, S. Sakuragi, M. Kawamura, and A. Adachi.** 1993. Cell-dependent requirement of human immunodeficiency virus type 1 Vif protein for maturation of virus particles. J. Virol. **67:**1663–1667.
25. **Shahabuddin, M., B. Volsky, M.-C. Hsu, and D. J. Volsky.** 1992. Restoration of cell surface CD4 expression in human immunodeficiency virus type 1-infected cells by treatment with a Tat antagonist. J. Virol. **66:**6802–6805.
26. **Smith, A. J., A. Srinivansan, M. L. Hammarskjold, and D. Rekosh.** 1993. Requirements for incorporation of $Pr160^{gag\text{-}pol}$ from human immunodeficiency virus type 1 into virus-like particles. J. Virol. **67:**2266–2275.
27. **Smith, S., M. Shatsky, P. Cohen, R. Wamke, M. Link, and B. Glader.** 1984. Monoclonal antibody and enzymatic profiles of human malignant lymphoid T-cells and derived cell lines. Cancer Res. **44:**5657–5660.
28. **Sova, P., and D. J. Volsky.** 1993. Efficiency of viral DNA synthesis during infection of permissive and nonpermissive cells with *vif*-negative human immunodeficiency virus type 1. J. Virol. **67:**6322–6326.
29. **Strebel, K., D. Daugherty, K. Clouse, D. Cohen, T. Folks, and M. A. Martin.** 1987. The HIV "A" (sor) gene product is essential for virus infectivity. Nature (London) **328:**728–7305.
30. **Tritch, R., Y. S. E. Cheng, F. H. Yin, and S. Erickson-Vitane.** 1991. Mutagenesis of protease cleavage sites in the human immunodeficiency virus type 1 *gag* polyprotein. J. Virol. **65:**922–930.
31. **Vaishnaw, Y. N., and F. Wong-Staal.** 1991. The biochemistry of AIDS. Annu. Rev. Biochem. **60:**577–630.
32. **Volsky, D. J., M. J. Potash, M. Simm, X.-Y. Ma, W. Chao, and M. Shahabuddin.** The human immunodeficiency virus type 1 *vif* gene: the road from an accessory to an essential role in human immunodeficiency virus type 1 replication. Curr. Top. Microbiol. Immunol., in press.
33. **von Schwedler, U., J. Song, C. Aiken, and D. Trono.** 1993. *vif* is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. J. Virol. **67:**4945–4955.
34. **Wang, C.-T., and E. Barklis.** 1993. Assembly, processing, and infectivity of human immunodeficiency virus type 1 Gag mutants. J. Virol. **67:**4264–4273.
35. **Willis, J. W., and R. C. Craven.** 1991. Form, function, and use of retroviral Gag proteins. AIDS **5:**639–654.